#10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Tadamitsu KISHIMOTO et al.

Serial No.: 08/817,084 Group Art Unit: 1816

Filed: April 7, 1997 Examiner: Vander Vegt, F

For CHRONIC RHEUMATOID ARTHRITIS THERAPY CONTAINING IL-6 ANTAGONIST AS EFFECTIVE COMPONENT

DECLARATION

Honorable Commissioner of Patent and Trademarks
Washington, D.C. 20231

Sir:

I, Masahiko Mihara, a citizen of Japan, residing at 135, Komakado 1-chome, Gotenba-shi, Shizuoka, Japan sincerely and solemnly declare:

THAT I am by profession an immunopharmacologist and that I graduated from postgraduate school of Gifu Pharmaceutical University, Department of Pharmacology in 1985. And since 1985, I have been researcher of Chugai pharmaceutical company. I obtained Ph. D. degree from graduate school;

THAT I am a coinventor of the present invention, and therefore completely familiar with the present invention and

the outstanding Office Action;

THAT in order to further demonstrate the enablement capability of the present invention, the following experiments were carried out under my direction and supervision.

Experiment 1. MRA (humanized anti-IL-6 receptor antibody) suppression of the onset of collagen-induced arthritis in monkeys

Bovine type II collagen (CII, Cosmo Bio, Tokyo, Japan) was dissolved overnight at 4°C in 0.1M acetic acid solution at a concentration of 4 mg/ml. The collagen solution was emulsified in an equal volume of complete adjuvant H37 Ra (DIFCO Laboratories). Monkeys (5 monkeys (one male and four females)/group) were immunized with 1 ml of the cold emulsion by 10 intradermal injections on the back and one injection into the base of the tail. Four weeks later, monkeys were boosted with CII by 20 intradermal injections on the back and one injection into the base of the tail.

The symptoms of arthritis were evaluated by the number of stiff joints, and swelling of 4 limbs. The number of stiff joints were counted visually. Briefly, stiffness of all joints of 4 limbs (64 joints) were investigated and scored, giving just one point for each joint which demonstrated stiffness without consideration of severity.

Swelling of 4 limbs was assessed by measuring the carpus and tarsus volume of both hands and feet by water immersion, and the sum of 4 limbs was normalized by body weight to calculate data for each animal. These above two examinations were performed in a blind manner.

Blood samples were collected every two weeks starting from just before the first immunization, and they were subjected to measurement of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

At the end of study, the histological evaluation was also carried out.

MRA (humanized anti-IL-6 receptor antibody) was dissolved in phosphate buffer containing 20 mM NaH_2PO_4 and 250 mM NaCl and administered intravenously once a week for 13 weeks. For the vehicle group, the same volume of phosphate buffer was administered in the same way.

All vehicle-treated monkeys revealed the clinical sign of arthritis 4 weeks after first collagen immunization. The number of stiff joints and swelling reached maximum at 8 weeks and 6 weeks, respectively. Thereafter, arthritis was quickly reduced. In contrast, the administration of MRA inhibited the onset of arthritis. The increase in the number of stiff joints and swelling rate was completely suppressed in four out of five monkeys of the MRA 10 mg/kg group

(Fig. A).

In the vehicle group, a dramatic increase in CRP was observed after 2 weeks and a clear increase of ESR after 4 weeks following the first immunization. These were transients, as in the case of arthritis symptoms, and the situation returned close to normal after 14 weeks. On the other hand, MRA inhibited the increase of these parameters almost completely (Fig. B).

A result of the histological examination is shown in Fig. C. As can be seen from Fig. C, no abnormalities in the joint were observed for monkeys treated with MRA, while synovial proliferation and articular cartilage degeneration and destruction were observed in the joint of monkeys not treated with MRA.

Experiment 2. MRA suppressed IL-6-induced CRP increase in monkey

Monkeys (2 monkeys (one male and one female/group) were intravenously administered once with MRA at a dose of 0 (vehicle) or 5 mg/kg. Subsequently, monkeys were subcutaneously injected with human recombinant IL-6 at a dose of 5 μg/kg once a day for 7 days.

Peripheral blood samples were collected daily day 0 to day 9, thereafter on days 11, 13, 15 and day 18, and they

were subjected to measurement of C-reactive protein (CRP).

MRA and human recombinant IL-6 was dissolved in saline containing 1% heat-inactivated cynomolgus monkey serum. For the vehicle group, the same volume of saline containing 1% heat-inactivated cynomolgus monkey serum was administered in the same way.

In the vehicle group, a dramatic increase in CRP was observed immediately after the first injection of IL-6. On the other hand, MRA inhibited the increase of CRP completely (Fig. D).

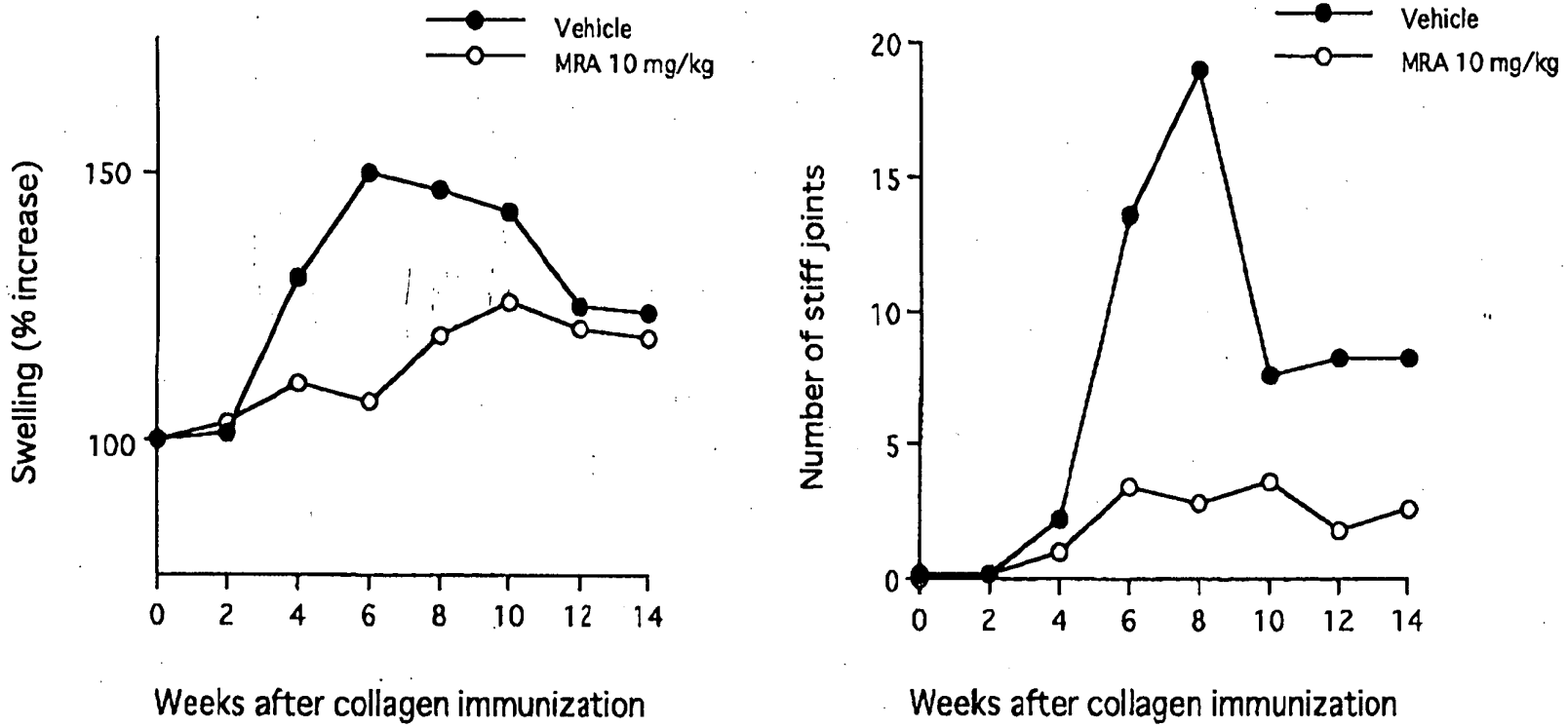
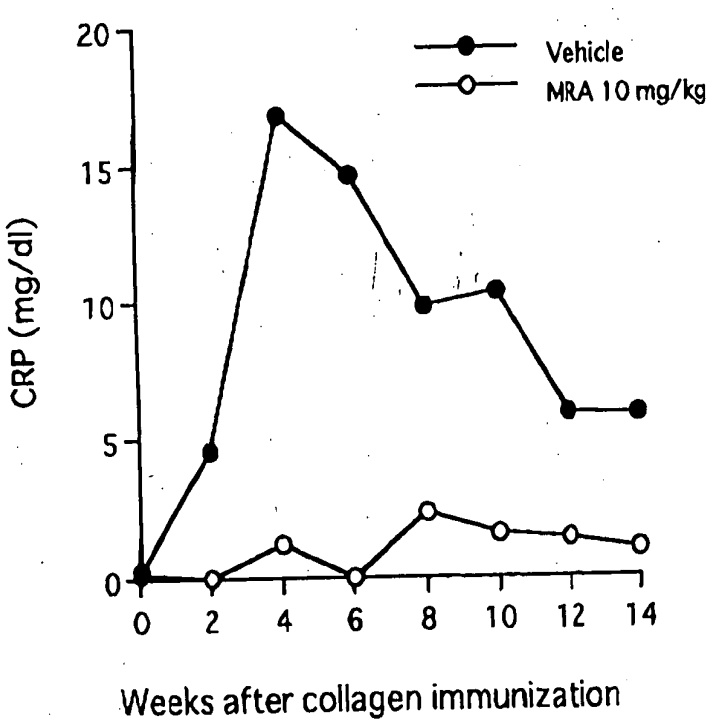

Vehicle
MRA 10 mg/kg
Swelling (% increase)
150
100
0
2
4
6
8
10
12
14
Weeks after collagen immunization
Vehicle
MRA 10 mg/kg
Number of stiff joints
20
15
10
5
0
0
2
4
6
8
10
12
14
Weeks after collagen immunization

Fig.A


CRP (mg/dl)
20
15
10
5
0
0 2 4 6 8 10 12 14
Weeks after collagen immunization
Vehicle
MRA 10 mg/kg

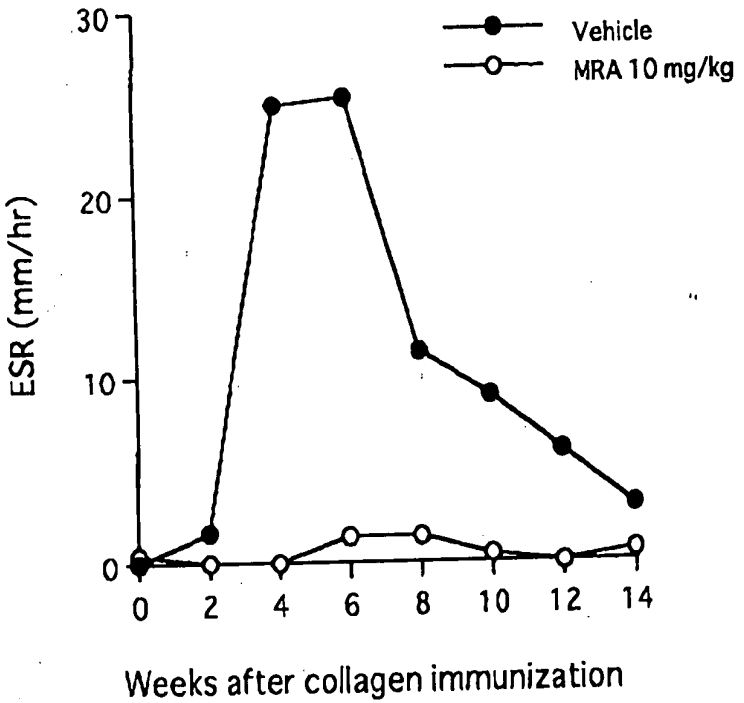

ESR (mm/hr)
30
20
10
0
0 2 4 6 8 10 12 14
Weeks after collagen immunization
Vehicle
MRA 10 mg/kg

Fig.B

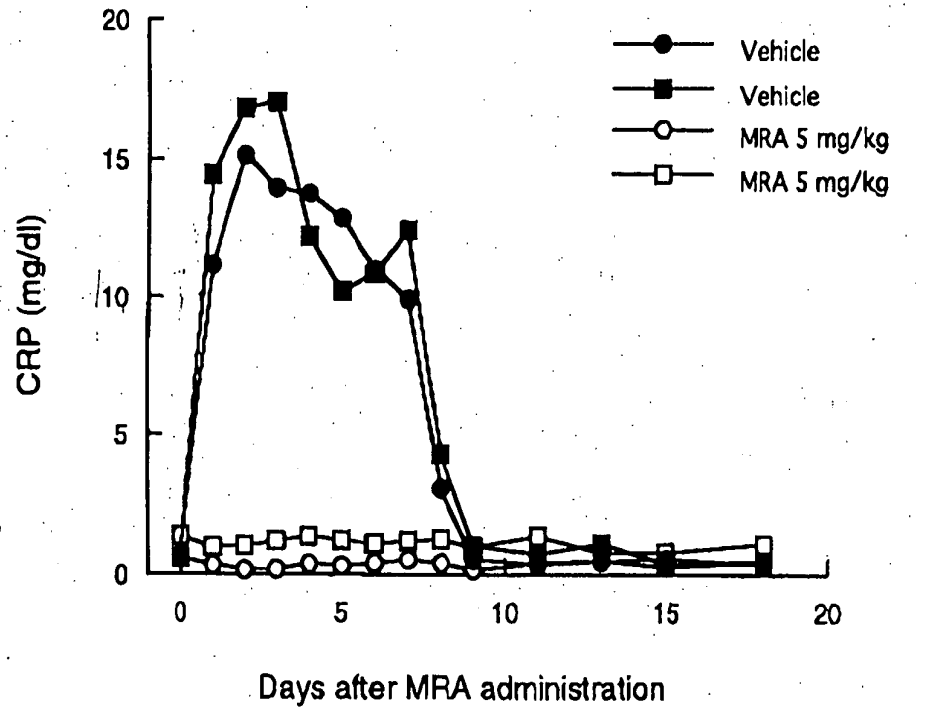

20
15
10
5
0
CRP (mg/dl)
0
5
10
15
20
Days after MRA administration
Vehicle
Vehicle
MRA 5 mg/kg
MRA 5 mg/kg

Fig.C

I, the undersigned declarant, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and; further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001, of Title 18, of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 4th day of November , 1997

Masahiko Mihara